

CLINICAL STUDY

Suboptimal effect of different vitamin D3 supplementations and doses adapted to baseline serum 25(OH)D on achieved 25(OH)D levels in patients with a recent fracture: a prospective observational study

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Abstract

Objective: Guidelines on the need for dose adaptation of vitamin D3 supplementation according to baseline serum 25(OH)D are inconclusive. The effects of increasing doses of vitamin D3 at lower baseline serum 25(OH)D values on the serum 25(OH)D after 4.2 and 11 months were determined in an observational study.

Design: A prospective observational study.

Methods: Out of 1481 consecutive women and men with a recent clinical fracture, 707 had a baseline 25(OH)D level < 50 nmol/l and were supplemented with increasing doses of vitamin D3 (400, 800, 1700, and ≥ 3500 IU/day) according to the lower baseline 25(OH)D. Final analysis was restricted to the 221 participants who had full follow-up data available for 11 months.

Results: Serum 25(OH)D ≥ 50 nmol/l was achieved in 57–76% of patients after 4.2 months and in 73–79% after 11 months. These percentages were similar for all doses ($P=0.06$ and $P=0.91$ respectively). The mean achieved 25(OH)D was similar for all dose groups (56.1–64.0 nmol/l after 4.2 months and 60.2–76.3 nmol/l after 11 months). With multivariate analysis, the increase in 25(OH)D (17 ± 32.0 after 4.2 months and 24.3 ± 34.0 nmol/l after 11 months) was dependent on the baseline 25(OH)D ($P<0.001$), not on supplementation dose, season, age, BMI, or gender.

Conclusions: The increase in serum 25(OH)D was significantly larger with higher vitamin D3 supplementation doses. However, this dose–effect response was mainly explained by the baseline 25(OH)D, not the supplementation dose, with a greater magnitude of response at lower baseline 25(OH)D concentrations. In 21–27% of patients, serum 25(OH)D3 levels did not reach 50 nmol/l after 11 months, at any dose. Further studies are needed to identify possible causes of suboptimal response such as non-compliance, undiagnosed malabsorption syndromes, or variability in cholecalciferol content of the vitamin D supplements.

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Introduction

Serum 25(OH)D is the most widely accepted indicator of vitamin D status (1). Despite the well-documented role of vitamin D in health, there is still debate on the optimal dose of vitamin D supplements needed to achieve a desired threshold of serum 25(OH)D concentration (e.g. loading doses or a constant dose) (2). There is no universally accepted threshold, neither in the USA where the Institute of Medicine report is more in favor of 50 than 75 nmol/l, nor in Europe where it is dependent

on countries' guidelines and different expert opinions (2, 3). Many factors have been reported to affect changes in serum 25(OH)D concentration using vitamin D supplements, such as sex, age, BMI, body composition, genetic factors, and variability in serum 25(OH)D assay methods (4). A dose-dependent effect of vitamin D supplements has been documented in healthy postmenopausal women with vitamin D insufficiency (serum level < 50 nmol/l) and for fracture prevention (5, 6). A dose of 800 IU/day was sufficient to increase the serum level of 25(OH)D above 50 nmol/l in 98% of

women (6). Other studies indicated a significant relation between baseline serum 25(OH)D and the response to vitamin D supplements, i.e. when using one dose of supplements, a higher increase is achieved when baseline levels are low than when baseline levels are normal (7). At present, there is little known about the effect of higher supplementation doses at lower baseline serum 25(OH)D levels on the increase and achieved serum 25(OH)D. We therefore conducted an observational study in patients who presented with a recent fracture and serum 25(OH)D level < 50 nmol/l to assess the effect of higher supplementation doses in patients with lower 25(OH)D levels.

Subjects and methods

Study design and population and data collection

This is a prospective, observational study designed to examine the effects of vitamin D3 supplementation on serum 25(OH)D levels in clinical practice in Caucasian patients of 50 years and older who presented with a recent clinical vertebral or non-vertebral fracture evaluated at the fracture liaison service (FLS) of the VieCuri Hospital Noord-Limburg (The Netherlands). At the FLS, after primary fracture care, a specialized nurse invited all patients to the fracture and osteoporosis outpatient clinic of the hospital for bone mineral density (BMD) measurement and laboratory tests. Fractures were classified according to Center *et al.* (8), into hip fractures, major fractures (vertebra, pelvis, distal femur, proximal tibia, multiple rib, and humerus), minor fractures (all remaining osteoporotic fractures, except fingers and toes), and finger and toe fractures. Patients with metastatic cancer in bone, high-impact multi-trauma, osteomyelitis, or failure of prosthesis were excluded. Patients who responded and agreed received a detailed questionnaire for the evaluation of clinical risk factors for fractures, medical history, medication, previous fractures, and calcium intake and were planned for dual X-ray absorptiometry (DXA) measurement and a blood test. A visit at the outpatient clinic was scheduled after completion of these tests. At this visit, height and weight were assessed, the questionnaire was evaluated and when necessary additional questions were asked, and physical examination was performed by a physician. If laboratory results were abnormal, additional investigations were performed for detailed evaluation of newly diagnosed disorders when necessary. Depending on the BMD results, calcium intake, and serum 25(OH)D levels, patients were treated with calcium and vitamin D3 supplements and anti-osteoporosis medication according to the Dutch guidelines for treatment of osteoporosis (9). Vitamin D3 supplementation was prescribed in all patients with a baseline 25(OH)D level < 50 nmol/l. The vitamin D3 supplementation dose was based on

serum 25(OH)D levels, where higher doses were started with lower serum 25(OH)D levels. Generally, patients with a 25(OH)D level between 25 and 50 nmol/l were supplemented with 800 or 400 IU vitamin D3, and those with a 25(OH)D level < 25 nmol/l were supplemented with higher doses of vitamin D3, especially for patients with a level < 15 nmol/l and/or secondary hyperparathyroidism.

Vitamin D3 was supplemented as liquid vitamin D3 solution (colecalciferol, 50 000 IU/ml as intermittent weekly, monthly, or bimonthly dose, or as daily tablets 400 and 800 IU/day or as Fosavance '5600' or '2800' per week up to the opinion of the physician). For this study the cumulative dose of vitamin D3 supplementation per month was recalculated to equivalent daily intake of vitamin D3 for each patient. All prescribed vitamin D preparations are approved by the European Medicines Agency and delivered by registered pharmacists.

According to the local protocol at the FLS and the Dutch guidelines for osteoporosis, a follow-up visit was planned based on clinical characteristics, such as the presence of metabolic bone disease or other co-morbidity, after initiation of anti-osteoporosis medication or other reasons based on the opinion of the clinician after ~ 4 and 12 months. During the follow-up visits, clinical parameters, current dietary and supplemental calcium intake, vitamin D3 supplementation, use of other medication, and additional laboratory tests were done for all patients.

Bone densitometry

BMD in the left or right hip and the lumbar spine was determined using DXA with the Hologic QDR 4500 (Hologic, Bedford, MA, USA). Diagnosis of osteoporosis was based on the WHO criteria for BMD (10), as provided by the manufacturer's database for women and men and which was based on the National Health and Nutrition Examination Survey III. *T*-score calculations were done for women using a female reference population and for men using a male reference population, as provided by the manufacturer. Patients were classified according to the lowest value of *T*-score at total hip, femoral neck, or lumbar spine: *T*-scores of < -2.5 (not 2.5) are considered in the osteoporotic range and *T*-scores between -1 and -2.5 (not 1 and 2.5) are considered osteopenic.

Laboratory measurements

Blood samples were collected at study visit baseline (a mean of 2–3 months after the fracture), after a mean of 4.2 months (visit 2), and after a mean of 11 months (visit 3). Basic laboratory tests included serum sodium, potassium, calcium, inorganic phosphate, albumin, creatinine, free tetraiodothyronine (fT_4), TSH, serum aminotransferases (aspartate aminotransferase and alanine aminotransferase), alkaline phosphatase, intact

plasma PTH (iPTH), 25(OH)D, and serum and urine protein electrophoresis for all patients. 25(OH)D vitamin D levels in serum were determined with the Architect i2000 immunochemistry analyzer (Abbott). The analytical performance of this method in comparison with the reference method has been described in the literature (11). The estimated glomerular filtration rate was calculated using the modification of diet in renal disease study group (MDRD) equation. The stages of chronic kidney disease (CKD) were defined according to the guidelines for CKD of the National Kidney Foundation (12). Primary hyperparathyroidism was diagnosed by hypercalcemia in the presence of inappropriately normal or elevated levels of iPTH (13, 14). Secondary hyperparathyroidism was defined as elevated plasma iPTH in combination with 25(OH)D < 50 nmol/l or CKD stage 3 or greater, or both. Hyperthyroidism was defined by TSH values < 0.50 mU/l with elevated fT_4 levels and subclinical hyperthyroidism by TSH values < 0.50 mU/l with normal fT_4 levels. Hypogonadism was defined as a clinical syndrome complex, which comprises both symptoms as well as biochemical testosterone deficiency. Testosterone deficiency was defined as a total testosterone level < 8 nmol/l (15). When inappropriate, additional evaluation followed.

Statistical analysis

All analyses were performed using SPSS for Windows (version 18.0, SPSS, Inc.). The results are presented as means \pm s.d. or percentages, if appropriate. Baseline characteristics were compared in each of the five groups (vitamin D supplementation: no dose, 400, 800, 1700, and ≥ 3500 IU/day) using ANOVA for continuous

variables and χ^2 tests for categorical variables. Box-Whisker was used to show the policy of vitamin D3 supplementation according to the baseline serum 25(OH)D. Absolute values of serum 25(OH)D were compared in each of the five groups using repeated measure ANOVA for continuous variables. Changes in 4.2 months and after 11 months were compared among vitamin D3 doses groups using both ANOVA and analysis of covariance to show the effect of confounding factors (baseline serum 25(OH)D). Serum vitamin D status was categorized based on 25(OH)D levels as being optimal (≥ 50 nmol/l), or having insufficiency (< 50 nmol/l) to find the distribution of patients with vitamin D insufficiency. We created a sub-group with a daily dose of 800 IU/day to test the association of changes in serum 25(OH)D and baseline serum 25(OH)D. Multiple linear regression analyses were carried out to study the predictors for the changes in serum 25(OH)D after 4.2 months and 11 months. The variables included in the model were age, gender, BMI, season, baseline serum 25(OH)D, and dose of supplementation. For all tests, a probability level < 0.05 was considered statistically significant.

Results

Baseline data

A total of 1481 patients (28.1% men and 71.9% women) with a mean age of 66.1 ± 10.4 were evaluated at the FLS. Of them, 30.6% were diagnosed with osteoporosis, 64.7% with osteopenia, and 4.8% had a normal BMD. Mean daily dietary calcium intake was 971 ± 356 mg/day and the baseline 25(OH)D level was 50.8 ± 25.5 nmol/l. According to the Center

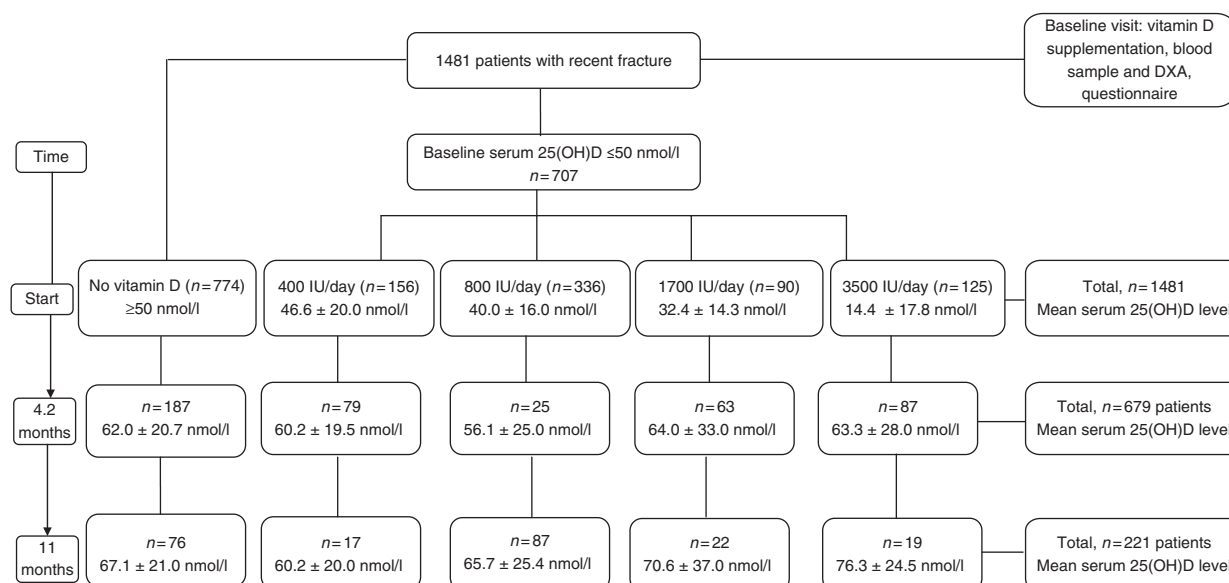


Figure 1 Study flowchart.

Table 1 Baseline characteristics for three populations.

	Total group (n=1481)	Patients with one follow-up visit after 4.2 months (n=707)	Patients with two follow-up visits after 4.2 and 11 months (n=221)
Age (years)	66.1±10.4	68.5±10.4	69.4±10.4
Male/female (%)	28.1/79.1	22.5/77.5	25.3/74.7
Weight (kg)	66.3±10.7	68.5±10.3	69.4±10.5
BMI (kg/m ²)	24.0±5.1	25.3±5.1	25.5±5.0
Calcium intake (mg/day)	970.8±356.8	962.3±375.5	976.6±350.6
Serum 25(OH)D (nmol/l)	50.8±25.5	41.5±24.7	42.8±22.7
Vitamin D status (%)			
<50 nmol/l	48.8	65.4	65.6
Serum PTH (pmol/l)	6.6±4.4	7.2±4.8	6.8±4.0
Serum calcium (mmol/l)	2.4±0.11	2.4±0.11	2.4±0.1
Serum phosphor (mmol/l)	1.2±0.18	1.2±0.16	1.2±0.17
Serum albumin (g/l)	41.0±3.1	40.6±3.1	40.7±3.0
Serum creatinine (μmol/l)	73.0±17.5	72.2±17.4	71.3±17.0
Serum TSH (U/l)	1.8±3.0	1.8±3.8	1.6±1.3

classification, 6.7% sustained a hip fracture, 35.6% a major fracture, 53.0% a minor fracture, and 4.7% a finger or toe fracture. Of all patients, 26.4% were diagnosed with at least one newly detected metabolic bone disease (24.7% men and 27.1% women), 9.9% with renal failure stage 3 or 4, 3.4% with subclinical or overt hyperthyroidism, 3.7% with primary hyperparathyroidism, 12.0% with secondary hyperparathyroidism due to vitamin D deficiency, 1.1% with secondary hyperparathyroidism due to renal failure, and in men 7.5% were diagnosed with hypogonadism.

Of the total group, 707 patients (47.7%; 159 men (24.9%) and 548 women (75.1%)) had a serum 25(OH)D level <50 nmol/l (Fig. 1). Vitamin D3 supplementation was started in 707 patients (159 men and 548 women) and calcium supplementation in 338 patients (86 men and 252 women) and 492 started with oral bisphosphonates. Of the total group of 1481 patients, 679 patients were evaluated after a mean of 4.2 ± 2.2 months and 221 at the second follow-up visit after 11 ± 4.4 months (Fig. 1). Baseline characteristics of patients that were evaluated at the initial, first, and second follow-up visit are presented in Table 1.

As vitamin D supplementation policy and serum 25(OH)D outcomes of the group that had one follow-up visit (at 4.2 months) and the group with two follow-up visits (4.2 and 11 months) were comparable at the first follow-up visit, we only present data of the 221 patients who were evaluated at all three visits during 11 months. A total of 76 patients did not receive vitamin D3 supplementation because they had a baseline 25(OH)D level ≥ 50 nmol/l. The vitamin D supplementation dose in patients with a baseline 25(OH)D level <50 nmol/l is shown in Fig. 2. There was a significant difference in age, serum (25(OH)D, and PTH between the different vitamin D3 dose groups (all $P < 0.05$; Table 2). There was no significant difference in baseline serum 25(OH)D levels or the percentage of patients with a 25(OH)D level <50 nmol/l

between the different fracture type groups or BMD groups (Table 2).

Serum 25(OH)D ≥ 50 nmol/l was achieved in 57–76% of patients after 4.2 months and in 73–79% after 11 months (Fig. 3), and 33.9% achieved a serum 25(OH)D ≥ 75 nmol/l. These percentages were similar for all supplementation doses ($P = 0.06$ and $P = 0.91$ respectively).

The achieved serum 25(OH)D was 60.2 ± 19.5 nmol/l with 400 IU vitamin D3/day, 56.1 ± 25.0 nmol/l with 800 IU/day, 64.0 ± 33.0 nmol/l with 1700 IU/day, and 63.3 ± 28.0 nmol/l for ≥ 3500 IU/day after 4.2 months and 60.2 ± 20.0 nmol/l with 400 IU/day, 65.7 ± 25.4 nmol/l with 800 IU/day, 70.6 ± 37.0 nmol/l with 1700 IU/day, and 76.3 ± 24.5 nmol/l for ≥ 3500 IU/day after 11 months (Fig. 4). After 4.2 and 11 months follow-up, mean serum

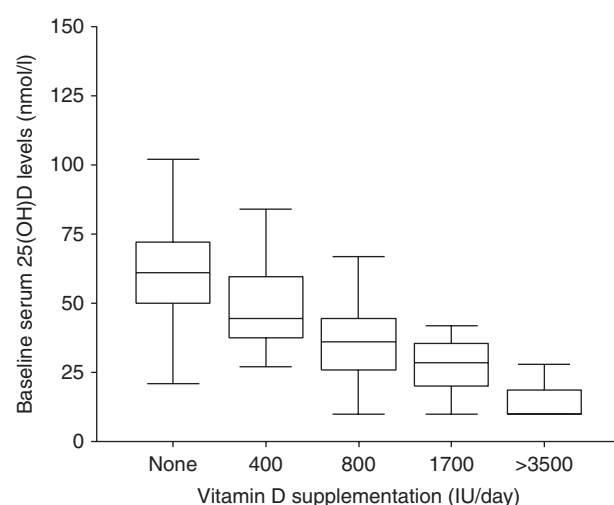


Figure 2 Vitamin D supplementation policy (IU/day) according to baseline serum 25(OH)D levels (nmol/l) in 221 patients with a recent fracture.

Table 2 Vitamin D supplementation policy, daily dietary calcium intake, laboratory results, and BMD status at baseline in 221 patients with a recent fracture who were evaluated during 11 months after starting vitamin D supplementation.

Characteristics	n=76	n=17	n=87	n=22	n=19	P value
Vitamin D supplementation dose	No	400 IU/day	800 IU/day	1700 IU/day	≥3500 IU	
Age (years)	66.3±9.8	67.0±10.3	71.5±10.0	71.7±12.7	71.3±10.5	0.013
Male/female	44.6/30.9	8.9/7.3	32.1/41.8	7.1/10.9	7.1/9.1	0.37
Weight (kg)	66.8±9.8	66.3±11.0	71.4±9.8	71.1±9.5	70.1±9.6	0.07
BMI (kg/m ²)	24.1±4.2	24.5±5.8	26.7±5.1	25.7±5.7	25.8±4.6	0.06
Daily dietary calcium intake (mg/day)	1030±362.6	936.2±380.2	947.0±351.3	938.7±303.8	966.7±30.6.0	0.63
Serum calcium (mmol/l)	2.4±0.09	2.4±0.08	2.4±0.09	2.4±0.1	2.4±0.08	0.62
Serum creatinine (μmol/l)	71.3±17.1	67.8±17.6	71.8±16.7	73.0±17.6	69.3±17.0	0.87
Serum 25(OH)D (nmol/l)	68.0±16.4	37.6±9.8	32.5±10.8	26.0±9.4	14.0±7.4	<0.001
Serum PTH (pmol/l)	5.8±2.8	6.8±3.3	7.3±3.9	8.8±7.0	6.5±3.2	0.022
Percentage of patients with osteoporosis/osteopenia/normal BMD	57.9/28.9/2.6	35.3/52.9/11.8	51.7/32.2/2.3	45.5/27.3/4.5	47.4/31.6/5.3	0.81

BMD, bone mineral density.

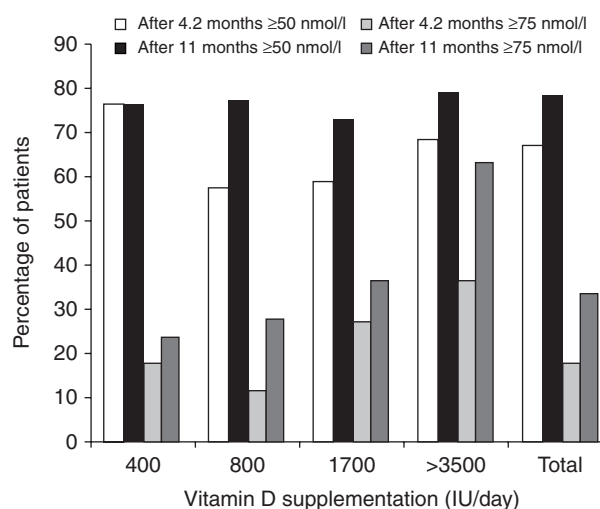
25(OH)D level was not significantly different between the patients who started and did not start vitamin D supplementation (58.7 ± 26.1 vs 62.0 ± 20.7 nmol/l after 4.2 months ($P=0.34$) and 67.2 ± 26.8 vs 67.1 ± 21.0 nmol/l after 11 months ($P=0.98$), Fig. 4A). Between-group comparisons in all vitamin D dose groups did not show significant differences at 4.2 and 11 months ($P=0.66$ and $P=0.058$ respectively).

The increase in serum 25(OH)D at 4.2 months was 22.6 ± 20.0 nmol/l with the 400 IU vitamin D/day, 23.6 ± 26.8 nmol/l with 800 IU/day, 38.0 ± 37.3 nmol/l with 1700 IU/day, and 49.4 ± 26.0 nmol/l with ≥ 3500 IU/day and at 11 months 22.6 ± 18.0 nmol/l with 400 IU/day, 33.1 ± 26.6 nmol/l with 800 IU/day, 44.6 ± 36.7 nmol/l with 1700 IU/day, and 62.3 ± 23.2 nmol/l with ≥ 3500 IU/day. Repeated-measures ANOVA showed a time-related interaction between vitamin D dose groups on changes in serum 25(OH)D ($P<0.001$). Between-group comparisons in changes of serum 25(OH)D levels were significantly higher after both 4.2 and 11 months with higher supplementation dose (P for trend ≤ 0.001 for both time intervals). However after controlling for baseline serum 25(OH)D, there was no significant difference between supplementation dose groups in changes of 25(OH)D levels after both 4.2 and 11 months ($P=0.43$ and $P=0.31$ respectively). There was a negative correlation between the increase in serum 25(OH)D and baseline 25(OH)D levels at both 4.2 and 11 months ($r=-0.44$ and $r=-0.55$ respectively, both $P<0.001$).

To identify the predictors of 25(OH)D response after supplementation, we conducted a stepwise linear regression model. The contribution of each variable to the model was shown in Table 3. After 4.2 and 11 months, baseline serum 25(OH)D accounted 68 and 64% of variations of the change in serum 25(OH)D in the group with 221 patients. The mean increase in 25(OH)D (17 ± 32.0 after 4.2 months and 24.3 ± 34.0 nmol/l after 11 months) was dependent

on the baseline 25(OH)D ($R^2=0.70$ and $R^2=0.70$, $P<0.001$ for both time intervals), not on supplementation dose, season, age, BMI, or gender.

As many guidelines on osteoporosis advocate a daily dose of 800 IU vitamin D/day, we analyzed the patients who received 800 IU vitamin D/day ($n=251$) separately. As mentioned above, 32.3% achieved a serum 25(OH)D level >50 after 4 months and 67.7% after 11 months. There was a negative correlation between the increase in serum 25(OH)D and baseline serum 25(OH)D levels ($r=-0.38$, $P<0.001$ after 4 months). After categorizing the 87 patients who were followed until 11 months into four subgroups according to the baseline serum 25(OH)D levels (<20 , $20-29$, $30-39$, and $40-49$ nmol/l), the change in serum 25(OH)D was significantly higher in patients with lower baseline

**Figure 3** Percentage of patients achieving serum 25(OH)D levels ≥ 50 or 75 nmol/l after 4.2 and 11 months according to different vitamin D supplementation doses.

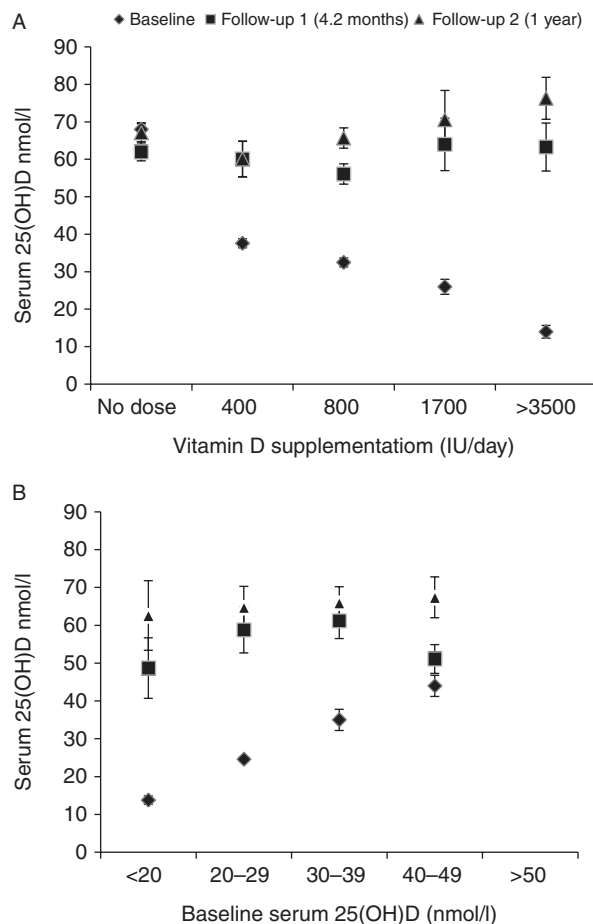


Figure 4 Serum 25(OH)D at baseline (diamonds), after 4.2 months (squares) and 11 months (triangles) follow-up in 221 (A) and 87 (B) patients with a recent fracture, according to daily vitamin D supplementation and baseline serum categories in a group with 800 IU/day vitamin D supplementation respectively.

levels ($P=0.003$). The mean achieved serum 25(OH)D levels were not significantly different between the four subgroups after 4 months ($P=0.33$) and after 11 months ($P=0.96$; Fig. 4B).

Discussion

In this prospective observational study in patients with a recent fracture who were treated in clinical practice, supplementation with higher doses of vitamin D3 in patients with lower baseline 25(OH)D levels increased serum 25(OH)D to a level ≥ 50 nmol/l in at least 57% of patients after 4 months and 73% after 11 months. The increase in serum 25(OH)D was significantly larger with higher vitamin D3 supplementation doses. However, based on the multivariate analysis, this dose–effect response was mainly explained by the baseline serum 25(OH)D and not the higher supplementation dose itself. In several studies, it has been reported that a higher vitamin D supplementation dose resulted in higher serum 25(OH)D levels (16, 17, 18, 19). However, in contrast to our study, the effect of different supplementation doses was investigated in subjects with 25(OH)D levels > 50 nmol/l and in groups with equal baseline 25(OH)D levels. Our observation, that lower baseline 25(OH)D concentrations resulted in a greater magnitude of response, was also previously reported (7, 20, 21, 22). Lips *et al.* reported a similar negative correlation between change in 25(OH)D level after supplementation and baseline 25(OH)D levels (20, 21). The policy in our study resulted in mean achieved 25(OH)D values that were comparable after 4 and 11 months for all doses. Apparently, using our supplementation policy, baseline serum 25(OH)D is the strongest predictor for the magnitude of 25(OH)D change, not the dose itself, even when using high supplementation doses.

Using a standard supplementation dose of 800 IU/day, as advocated in many guidelines on osteoporosis and fracture prevention (23, 24), the percentage of patients reaching a serum 25(OH)D level ≥ 50 nmol/l were similar when using higher doses, at any baseline serum 25(OH)D. This result confirms that baseline serum 25(OH)D is the most important predictor of response to vitamin D3 supplementation. Therefore, if the aim is to achieve a serum 25(OH)D ≥ 50 nmol/l, as in our study, higher vitamin D supplementation doses than 800 IU/day are not needed (6, 7, 24). However, if

Table 3 Multiple linear regression analysis for assessing the predictors of vitamin D supplementation response in 221 subjects after 4.2 and 11 months' follow-up.

	Change after 4.2 months				Change after 11 months			
	B	S.E.M.	β	P value	B	S.E.M.	β	P value
Constant	76.690	16.766		<0.001	45.401	18.96		0.018
Vitamin D dose (IU/day)	0.001	0.001	0.058	0.39	0.002	0.001	0.080	0.241
Baseline serum 25(OH)D (nmol/l)	−0.905	0.091	−0.68	<0.001	0.969	0.103	−0.647	<0.001
Season	0.068	1.562	0.002	0.96	2.230	1.790	0.071	0.215
Age (years)	0.453	0.300	−0.153	0.133	0.597	0.384	0.178	0.122
BMI (kg/m ²)	−0.304	0.633	−0.048	0.638	−1.426	0.865	−0.201	0.101
Gender	8.506	4.841	0.122	0.081	5.021	5.476	0.064	0.361

the aim is to achieve a serum 25(OH)D ≥ 75 nmol/l, higher doses may be needed. Indeed, a threshold ≥ 75 nmol/l was only achieved in one-third of the patients taking supplementation doses of up to 1700 U/day, and in two-thirds of patients using doses of 3500 IU/day or more.

Quite surprisingly, $> 20\%$ of patients in this study did not reach the threshold of 50 nmol/l, at any dose. One possibility for non-response is low compliance (25), which is a problem in many chronic diseases in clinical practice, including osteoporosis and fracture prevention (26). Another possibility is malabsorption, such as in celiac disease (27). In this study, however, we did not systematically check compliance and investigate whether patients had a malabsorption syndrome, so we do not know to what extent these aspects may have contributed to this finding. In addition, Leblanc *et al.* (28) have recently reported that the cholecalciferol content of compounded vitamin D supplements was highly variable (23–146%), even within the same formulation and dose. Another possibility is the genetic background. Recently it has been emphasized that genetic make-up of subjects may be important with regard to the response to vitamin D supplementation or diet. This individual variability may be, at least in part, explained by vitamin D receptor polymorphisms, vitamin D-binding protein, or other genetic determinates of serum 25(OH)D (29, 30, 31). Additionally, it was reported that there are four different types of 25 hydroxylases (32). Holick *et al.* reported that these enzymes most likely have different affinities for vitamin D and have different levels of negative feedback regulation by the serum 25(OH)D concentration. Thus, circulating 25(OH)D concentrations in response to vitamin D may be influenced by the baseline 25(OH)D concentration (33). Studies reported that even with good compliance, there is large variability in the response of 25(OH)D during vitamin D3 supplementation, and pointed out the prevalence of non-response patients in their populations (34), as also reported by Gallagher *et al.* (6). Considering the high prevalence of vitamin D deficiency in the elderly and assuming the same prevalence of 'non-responders' among the elderly population, a huge number of people may not benefit sufficiently from intake of the usually recommended amount of vitamin D. Given these considerations, follow-up measurements are helpful to detect suboptimal 25(OH)D levels and to adjust supplementation management.

Our study has several limitations. Firstly, this was an observational, non-randomized study. Secondly, we did not assess compliance. Thirdly, we did not assess baseline dietary vitamin D intake or a change in intake during the study. However, it is well documented that dietary vitamin D intake is low in The Netherlands (35). Fourthly, the various doses of vitamin D were in different formulations.

In conclusion, baseline serum 25(OH)D levels, but not supplementation dose, determined the response to vitamin D supplementation. The increase in serum 25(OH)D levels was higher in patients with lower baseline levels. In 21–27% of patients, serum 25(OH)D3 levels did not reach 50 nmol/l after 11 months, at any dose. Further studies are needed in order to identify possible causes of suboptimal response such as non-compliance, undiagnosed malabsorption syndromes, or variability in cholecalciferol content of the vitamin D supplements.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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P P M M Geusens and J P W van den Bergh organized the study concept and design. S Shab-Bidar, P P M M Geusens, and J P W van den Bergh took responsibility for the integrity of the data and the accuracy of the data analysis. S Shab-Bidar, J P W van den Bergh, and P P M M Geusens acquired, analyzed, and interpreted the data. S Shab-Bidar, P P M M Geusens, J P W van den Bergh, and S P G Bours drafted the manuscript. S Shab-Bidar, P P M M Geusens, and J P W van den Bergh undertook statistical analysis. S Shab-Bidar, S P G Bours, P P M M Geusens, J P W van den Bergh, R Y van der Velde, and M J W Janssen critically reviewed the manuscript for important intellectual content.

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